[CANCER RESEARCH 49, 1322–1326, March 1, 1989]

Case-Control Study of Colorectal Cancer and Fecapentaene Excretion[1]

Mark H. Schiffman,[2] Roger L. Van Tassell, Adam Robinson, Lee Smith, James Daniel, Robert N. Hoover, Roselyn Weil, Jeanne Rosenthal, Padmanabhan P. Nair, Samuel Schwartz, Hugh Pettigrew, Stephen Curiale, Gerald Batist, Gladys Block, and Tracy D. Wilkins

Epidemiology and Biostatistics Program, Division of Cancer Etiology, National Cancer Institute, Bethesda, Maryland 20892 [M. H. S., R. N. H., R. W., H. P.]; Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 [R. L. V., T. D. W.]; National Naval Medical Center, Bethesda, Maryland 20814 [A. R., S. C.]; George Washington University Hospital, Washington, DC 20037 [L. S.]; Walter Reed Army Medical Center, Washington, DC 20307 [J. D.]; Westat, Inc., Rockville, Maryland 20850 [J. R.]; Lipid Nutrition Lab, United States Department of Agriculture, Beltsville, Maryland 20705 [P. P. N.]; Minneapolis Medical Research Foundation, Minneapolis, Minnesota, 55404 [S. S.]; Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20892 [G. Ba.]; and Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, Maryland 20892 [G. Bl.]

ABSTRACT

The fecapentaenes are potent mutagens found in high concentrations in the stools of some individuals. These compounds are produced *in vivo* by common species of the colonic microflora, from precursors of unknown origin. The fecapentaenes have been postulated to increase the risk of colorectal cancer. To test this hypothesis, we measured fecapentaene excretion in 69 patients with adenocarcinoma of the colon or rectum, newly diagnosed at three Washington, DC area hospitals. The cases were compared with 114 surgical controls, frequency matched to the cases on age, sex, and hospital. We attempted to measure fecapentaene excretion 4 times for each subject: before surgery; and at 1 mo; 3 mo; and 6 mo following surgery. Contrary to our study hypothesis, we found fecapentaene excretion during the four study periods to be similar or even lower in cases compared to controls. An indirect measurement of fecapentaene precursors also tended to be lower in cases. The case-control differences could not be explained as effects of bleeding or of the colorectal diagnostic workup, which was assessed in a separate group of 86 patients. We conclude from these data that the excretion of fecapentaenes does not increase the risk of colorectal cancer, at least when measured near the time of diagnosis.

INTRODUCTION

The fecapentaenes are potent mutagens excreted in the feces of humans and some animals (1, 2). These ether-linked lipids are produced by colonic *Bacteroides* species from an unidentified group of precursor compounds (3). The fecapentaenes are highly genotoxic in a variety of bacterial and mammalian *in vitro* assays, accounting for much of the mutagenicity detected by *Salmonella typhimurium* tester strains TA98 and TA100 in organic extracts of feces from North American subjects (4–9). Since fecal mutagenicity has been observed to be increased in populations at elevated risk of colorectal cancer, the discovery of the fecapentaenes has raised the possibility of identifying colorectal carcinogens of etiological and screening importance (10–12).

To test the hypothesis that increased fecapentaene excretion causes colorectal cancer, we conducted a case-control study in Washington, DC. As part of the investigation, we addressed two methodological questions viewed as critical to the case-control comparison of fecapentaene excretion: (*a*) Does the colorectal diagnostic workup (undergone by cases but not by controls) affect fecapentaene excretion and thus invalidate the comparison? and (*b*) Does fecapentaene excretion in cases change as a result of hospitalization, surgery, and recovery? The results of both the methodological investigation and the case-control comparison are presented here.

Received 6/24/88; revised 11/3/88; accepted 11/14/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This research was supported in part by National Cancer Institute Contract FOD-0653.

[2] To whom requests for reprints should be addressed.

SUBJECTS AND METHODS

Study Population. We recruited patients newly diagnosed with adenocarcinoma of the colon or rectum, seen during the study period (April 1985 to June 1987) by the clinical coworkers at three Washington, DC, area hospitals: National Naval Medical Center; Water Reed Army Medical Center; and George Washington University Hospital. Since we planned to measure fecapentaene excretion both prior to and following treatment, cases scheduled for immediate surgery following diagnosis and those residing outside the Washington, DC, metropolitan area were not eligible ($n = 26$). Of the 162 presumably eligible cases, 21 (13%) were not reached before surgery, and 49 (30%) refused to participate due to the intensive study demands. An additional 14 cases dropped out before collecting a stool sample, leaving 78 participating cases (48%). Nine of these participants were found to have adenomatous polyps or nonneoplastic bowel conditions following surgery or pathology review and were excluded from analysis. As a result, 69 cases comprised the analytical study group. For controls, we recruited Washington, DC, area residents awaiting elective surgery for nononcological, nongastrointestinal conditions at the three study hospitals. Controls at each hospital were frequency matched to cases on age and sex. We approached 315 potential controls and found 277 to be eligible based on the additional criteria of Washington, DC, area residency and availability for follow-up. Of the 277 eligibles, 55 (20%) were not reached before surgery, 95 (34%) refused to participate, and 13 (5%) dropped out before collecting a stool, leaving 114 participants (41%). The distribution of control surgeries was as follows: 62 orthopedic or neurosurgical procedures (54%); 31 hernia repairs (27%); 16 vascular procedures (14%); and 5 other general surgeries, primarily of the thyroid (5%).

We wished to study the effects of the diagnostic workup for colorectal cancer on fecapentaene excretion, but it proved impossible to identify efficiently the cases prior to their diagnostic procedures, which included sigmoidoscopy, barium enema, and colonoscopy. Therefore, we recruited a separate group of 86 subjects undergoing one or more of these procedures as routine screening or for symptoms compatible with colorectal cancer (such as rectal bleeding, constipation/diarrhea, unexplained anemia). This investigation was called the "methods study" to distinguish it from the case-control comparison. Forty of the subjects in the methods study underwent cleansing enemas plus sigmoidoscopy, 21 had barium enemas, and 32 had p.o. bowel preparation followed by colonoscopy. (Seven of the individuals had more than one diagnostic procedure.) Two of the 86 subjects in the methods study proved to have colorectal cancer and were subsequently included in the case-control comparison as well.

Collection Procedures. Subjects in the methods study were asked to collect two 2-day stool specimens at home, one before and one shortly after the diagnostic procedure. The follow-up collection ranged from 1 to 74 days after the procedure (median, 12 days). In the case-control study, subjects were asked to collect four 2-day stool samples at home. The first sample was collected before hospitalization and treatment, which usually involved surgery. The three follow-up collections were scheduled at 1, 3, and 6 mo following surgery. Cases not undergoing surgery were asked to donate a single pretreatment sample. Subject participation varied for each collection period, so that the full group of 69 cases and 114 controls did not participate in each collection.

For each 2-day collection, the subjects received a styrofoam chest filled with dry ice. They collected stool into a plastic container held by

a collection bonnet placed on the toilet rim, then placed the container immediately into the dry ice chest. Subjects were shown how to use the collection kits to avoid urine contamination, and this proved not to be a problem. Stools not collected during the 2-day period were recorded, and this list was transported with the frozen stools to the lyophilization laboratory (United States Department of Agriculture).

Stools were freeze-dried in their individual containers without ever thawing. The lyophilate was pooled, mixed for the entire 2-day collection, and then stored at −40°C or colder in sealed, air-tight containers. Aliquots of the samples were sent in screw-top vials in batches on dry ice to the testing laboratory at Virginia Polytechnic Institute (Blacksburg, VA).

We conducted two different fecapentaene assays. Duplicate 1-g samples of the lyophilized stool were placed in 25- x 150-mm tubes and stoppered under argon. One sample was stored at −80°C to be assayed directly for fecapentaenes. We incubated the other sample with a fecapentaene-producing strain of *B. thetaiotaomicron* (VIP strain 5482) and measured the amount of fecapentaenes produced from already formed precursors over 4 to 5 days. This length of incubation is known to be sufficient to exhaust the available precursors in the sample. The incubated measurement thus represents already formed fecapentaenes as well as those newly processed from precursors, and it is considered to be an indirect assay of the precursors (13). Specifically, the sample incubated to measure precursors was (*a*) inoculated with 5 ml of "standard inoculum" as previously described (2), (*b*) incubated at 37°C for 4 to 5 days, (*c*) frozen at −80°C, (*d*) relyophilized, and (*e*) stored at −80°C. All inoculations, mixing, and incubations were done anaerobically under CO_2. The unincubated and incubated freeze-dried samples were extracted and analyzed for fecapentaenes by HPLC[3] as previously described. Briefly, freeze-dried samples were extracted with acetone supplemented with the antioxidant BHT. Each extract was vacuum filtered, evaporated under vacuum at 60°C, stoppered under argon, and placed in ice. The evaporated extracts were (*a*) resuspended in 0.5 ml of prechilled HPLC solvent, (*b*) filtered through an Acrodisc LC-13 filter (0.5 μm) into 1-ml septum-capped vials, and (*c*) sealed under argon.

We determined the concentration of total fecapentaenes in each extract on a Waters liquid chromatograph using a radial compression module and 8-mm (5 μm) silica cartridges, with chloroform:isopropanol (95:5) containing 50 g/ml of BHT as an antioxidant. The flow rate was 2 ml/min. The HPLC was calibrated using dilutions of known concentrations of synthetic fecapentaene-12; the peak areas of these standards were then correlated with those of the experimental samples. Absorbance was monitored at 365 nm on a Waters 440 UV detector (AUFS 0.05), and peak areas were integrated on a Hewlett Packard 3390A recording integrator. The reading corresponded to "total fecapentaenes," without separation of fecapentaene-12 and fecapentaene-14 (14). Assays were performed in batches as the samples were collected, without knowledge of which sample derived from which individual.

In additional laboratory measurements, selected case (n = 38) and control (n = 50) presurgical specimens were analyzed by HemoQuant for fecal hemoglobin (15), to assess the effect of bleeding, which was common in cases, on fecapentaene levels. To verify that no sample degradation or laboratory drift had occurred over the 2-yr study period, we selected every 20th sample submitted throughout the investigation and resubmitted these in a masked batch at the end. The correlation between first and replicate measurements was 0.91 for the direct and 0.94 for the incubated fecapentaene measurements, and there was no trend to indicate laboratory or storage problems.

Statistical Analyses. Fecapentaene values were nonnormally distributed. Simple statistical transformation was not possible, so nonparametric statistical methods were used (16). Correspondingly, median values are presented in the tables, since the means were excessively influenced by a few very high measurements at the tails of the distributions. For correlational analyses, the Spearman rank correlation was used. For paired analyses (*e.g.*, measurements for the same individual, before *versus* after barium enema or surgery), the Wilcoxon paired-sample test was chosen. For unpaired comparisons (*e.g.*, cases *versus* controls), the Mann-Whitney two-sample test was used. For all statistical manipulations, samples with no detectable fecapentaenes were assigned a value of 10 ng/g (the threshold of detection is 20 ng/mg). Samples with detectable fecapentaenes but nonintegrable peaks (less than 100 ng/g) were assigned a value of 60 ng/g, the midpoint of the nonintegrable range.

[3] The abbreviations used are: HPLC, high-performance liquid chromatography; BHT, butylated hydroxytoluene; FP, fecapentaenes.

RESULTS

To estimate "normal" fecapentaene excretion among subjects without apparent gastrointestinal abnormalities, we first examined fecapentaene measurements in the control group, among the 113 men and women who collected a stool sample before their surgeries for nongastrointestinal, nononcological conditions. As shown in Table 1, about 33% of the controls excreted very low levels of fecapentaenes, less than the integrable measurement threshold of 100 ng of FP/g of lyophilized stool. Another 41% of the controls excreted moderate fecapentaene levels, from 100 to 999 ng of FP/g. The upper 26% of the controls excreted over 1,000 ng of FP/g, with the uppermost 5% of the group over 5,000 ng of FP/g. As expected, incubated values, representing the sum of fecapentaene and precursor concentrations, were always equal to or greater than the direct measurements. On average, incubation increased fecapentaene concentration about 10- to 20-fold. As a result, only 12% of the controls had incubated measurements less than 100 ng of FP/g. Eighty % had values over 1,000 ng of FP/g, and 31% had levels over 10,000 ng of FP/g.

The direct and incubated fecapentaene measurements were highly correlated in the 730 stools tested (Spearman r = 0.69, P = <0.01). Only direct fecapentaene measurements are presented here because of this high correlation, except in the few instances where the incubated results suggested slightly different conclusions.

Methods Study. Subjects in the methods study excreted fecapentaene levels roughly comparable to the control group described above. Specifically, the median preprocedure fecapentaene level in the methods study was 432 ng/g of dry stool, and about two-thirds of the subjects had fecapentaene levels above the measurement threshold of 100 ng/g. The paired results from the methods study are given in Table 2. Each subject's measurement before a diagnostic procedure was subtracted from the measurement afterward, to estimate the effect of the test on fecapentaene excretion. As shown in Table 2, directly measured fecapentaene excretion was not affected significantly

Table 1 *Direct and incubated FP measurements among 113 controls, before surgery*

FP concentration (ng/g dry stool)	Control distribution	
	Direct measurement (%)	Incubated measurement (%)
<100	33	12
100–999	41	8
1,000–4,999	21	28
5,000–9,999	3	21
10,000+	2	31

Table 2 *Effects of colorectal diagnostic procedures on FP excretion*

Procedure	No. of subjects	Median effect[a] (ng/g)	P^b
Cleansing enema/sigmoidoscopy	40	−50	0.70
Barium enema	21	0	0.36
Bowel prep/colonoscopy	32	0	0.89

[a] Fecapentaene level after procedure minus fecapentaene level before.
[b] P values from Wilcoxon paired-sample test.

by any of the diagnostic procedures. Incubated fecapentaene values did seem to increase following cleansing enema/sigmoidoscopy (median rise of 1206 ng/g, $P = 0.04$). This increase was apparent even in the few subjects whose postprocedure measurements were delayed up to 2 mo following sigmoidoscopy. However, the more invasive procedures, barium enema and colonoscopy, did not significantly affect incubated fecapentaene measurements.

Case-Control Comparison. Cases and controls differed slightly with regard to demographic features, indicating that frequency-matching was imperfect. The median age of the cases was 65 yr compared with 60 yr in controls; 67% of the cases were males, compared with 54% males among the controls. Race, which was not a matching factor, also varied slightly between the two groups. Seventy-seven % of the cases were white, compared with 88% of the controls.

Of the four requested stool collections, the cases completed a mean of 2.6 collections, and the controls completed a mean of 2.8. Other parameters of stool sampling were also quite similar in the two groups, including numbers of stools contributing to each collection, as well as time intervals from presurgical collection to surgery, and from surgery to each of the three follow-up collections, which sometimes varied considerably from the planned 1-3-6-mo schedule (ranges are given in the tables).

The effects of surgery and recovery on fecapentaene excretion are shown in paired analyses for cases and controls separately in Table 3. In both groups, when presurgical measurements were considered as baseline, fecapentaene excretion tended to increase during follow-up. The rise in postsurgical values was especially pronounced in cases, reaching marginal statistical significance at 3 mo postsurgery ($P = 0.04$) and 6 mo postsurgery ($P = 0.06$).

When we divided the cases into three crude "stages," *in situ*, locally invasive, and metastatic, we observed the same postsurgical pattern of increasing FP excretion in all three stages. Separating the cases into right-sided, left-sided, and rectal lesions revealed the same pattern, which was also persistent when the cases were divided by age group (55 or less, 56–65, and over 65), sex, race, and hospital.

For incubated fecapentaene values, the general pattern was the same, but the rise at 3 and 6 mo in cases was weaker, while the increase among controls was stronger, reaching statistical significance when the values for controls at 3 mo were compared with their baseline ($P = 0.03$).

In Table 4, the results of the case-control comparison are presented. These indicate that cases excreted lower fecapentaene levels than controls. The time trends within the case and control groups shown in Table 3 were also reflected in the case-control comparisons, which gave stronger or weaker results depending on the period of comparison. Specifically, cases excreted significantly lower fecapentaene levels before surgery ($P < 0.01$) and at 1 mo following surgery ($P = 0.03$). Before surgery, only 40% of cases had integrable measurements over 100 ng of FP/g (compared to 67% in controls, see Table 1), and only 14% had values over 1000 ng/g (compared to 26% among controls). As case fecapentaene levels rose at the 3- and 6-mo follow-ups, they no longer had significantly lower levels than controls. For the incubated values, however, since case levels did not rise strongly, cases were substantially lower at all four comparison periods (presurgery, $P < 0.01$; 1 mo, $P < 0.01$; 3 mo, $P = 0.14$; 6 mo, $P = 0.07$). For both direct and incubated fecapentaene values, the overall lower levels of fecapentaene excretion in cases were seen regardless of cancer stage or site. Similar results were seen when we restricted the analysis to the 29 cases and 60 controls with complete (four) stool collections.

Table 3 *Effects of surgery and recovery on fecapentaene excretion in colorectal cancer patients and controls*

	Change compared to presurgical values[a]					
	Cases			Controls		
Sampling period	*n*	Median change	*P*[b]	*n*	Median change	*P*[b]
Presurgery		Baseline			Baseline	
One mo follow-up (13–69 days postsurgery)	35	0	0.87	68	0	0.33
Three mo follow-up (78–150 days postsurgery)	36	+234	0.04	67	0	0.43
Six mo follow-up (153–223 days postsurgery)	35	+50	0.06	65	+50	0.17

[a] Fecapentaene measurement at follow-up minus fecapentaene measurement before surgery.
[b] *P* values from Wilcoxon paired-sample test.

Table 4 *FP excretion in colorectal cancer patients versus controls, at four times around surgery*

	Cases		Controls		
Sampling period	*n*	Median FP (ng/g)	*n*	Median FP (ng/g)	*P*[a]
Presurgery	64	60	113	276	<0.01
One mo follow-up (13–69 days postsurgery)	39	60	69	184	0.03
Three mo follow-up (78–150 days postsurgery)	38	360	68	274	0.80
Six mo follow-up (153–223 days postsurgery)	37	253	66	540	0.25

[a] *P* values from Mann-Whitney two-sample test.

In an attempt to explain the lower fecapentaene excretion in cases compared with controls, we focused on the presurgical period, when participation was highest and the case-control difference was strongest. First we considered possible demographic influences. Among the controls, fecapentaene excretion decreased with age ($P = 0.06$ for 3 age groups, Mann-Whitney two-sample test) and was higher in men than in women ($P < 0.01$). However, these influences on fecapentaene excretion did not confound the case-control comparison. Lowered presurgical values in cases were seen in direct and incubated measurements in both sexes, in three age groups (≤55, 56–65, 66+), in white and nonwhite patients, in all three hospitals, and in patients with varying degrees of participation (those eventually providing one, two, three, or four stool collections). Cases had lower presurgical fecapentaene excretion than any diagnostic subgroup of the controls (orthopedic, vascular, hernia repair) and were also lower than the participants in the methods study (median direct measurement, 432 ng of FP/g).

The lower values in cases could not be explained by colorectal bleeding. The effects of colorectal bleeding were considered especially important as a potential confounding factor, since colorectal cancer patients were likely to be bleeding substantially more often than controls. In fact, as shown in Table 5, 20 (53%) of 38 cases tested by HemoQuant had evidence of substantial blood in their presurgical sample, compared with only 7 (14%) of 50 controls. However, fecapentaene excretion (direct and incubated) was lower in cases in both the bleeding and nonbleeding groups (Table 5).

DISCUSSION

Fecal mutagenicity is elevated in populations at high risk of colorectal cancer (10, 12), and the fecapentenes are the predominant fecal mutagens excreted by North American subjects (4). However, contrary to our study hypothesis, fecapentaene excre-

Table 5 *FP excretion in 38 colorectal cancer patients versus 50 controls, by bleeding status*

	n	Median FP (ng/g)	*P*[a]
Bleeding[b]			
Cases	20	60	0.25
Controls	7	267	
Not bleeding			
Cases	18	82	0.04
Controls	43	351	

[a] *P* values from Mann-Whitney two-sample test.
[b] Bleeding defined as greater than 7 mg of heme per g of dried stool by HemoQuant.

tion does not appear to pose a risk of colorectal cancer, at least as measured in a case-control study near the time of cancer diagnosis. Our cases had generally lower fecapentaene excretion than controls, considering either median FP values or the percentage of subjects with especially elevated levels.

Of concern in a case-control approach was that colorectal cancer itself, or its diagnosis and treatment, could affect the measurements. In particular, we recognized that the diagnostic workup for colorectal cancer might invalidate the case-control comparison, since fecapentaenes are produced by bowel flora which could be perturbed by barium enemas or cleansing regimens preceding endoscopy. We observed, however, no strong effect of the diagnostic workup on fecapentaene excretion. The finding of an increase in fecapentaene precursors following cleansing enema and sigmoidoscopy is probably a chance finding, since the more invasive diagnostic procedures showed no effect. We conclude from this methods study that diagnostic workup cannot explain the lower fecapentaene levels seen in colorectal cancer cases, although the statistical power of the methods study was not large enough to rule out the possibility of small effects.

Our second concern was that the timing of the case-control comparison might influence the results. In most case-control studies, cases are identified from medical records following treatment, which for colorectal cancer may include bowel resection with preparatory antibiotics, as well as adjunctive chemotherapy or radiation. We reasoned that fecapentaene excretion might be profoundly changed by these procedures and, thus, we measured cases before treatment (which usually was limited to surgery) as well as afterward. We also followed controls after their nongastrointestinal surgeries, to check for general effects of hospitalization and recovery. The results indicate that the timing of the case-control comparison is important. Both case and control measurements tended to rise following surgery. Cases excreted much lower levels of fecapentaenes than controls in the periods before hospitalization and immediately after surgery, but the difference was not significant later in recovery. However, incubated fecapentaene levels, representing fecapentaene precursors, were persistently lower in cases, regardless of sampling period. Thus, a general reduction was seen in cases, involving fecapentaene precursors as well as fecapentaene production itself.

The lowered fecapentaene levels in cases were clearly not due to colorectal bleeding. As another possible explanation for the case-control difference, we are currently examining dietary data collected from all subjects, to assess whether cancer-related dietary changes might have lowered fecapentaene excretion in cases (17). We have ruled out the possibility that alterations in recent fat intake could produce this effect (18).

It could be argued that measuring fecapentaene excretion near the time of diagnosis, either before or after surgery, is too late. Perhaps our colorectal cancer patients originally had elevated fecapentaene excretion, which fell as disease progressed. Two pieces of evidence argue against this objection. (*a*) When the case group was divided by stage, the nine patients with minimal, *in situ* cancers had lower levels prior to surgery compared with controls. (*b*) Individuals with adenomatous polyps may also demonstrate lowered fecapentaene excretion, as seen in a cross-sectional autopsy study reported by Correa *et al.* (19). They used *Salmonella* tester strain TA100, a mutagenicity assay known to detect fecapentaenes, and found lower mutagenicity in subjects with adenomatous polyps than in those with no polyps. Thus, if fecapentaene excretion is truly increased in subjects prone to colorectal neoplasia, this association, opposite to what we observed, might be seen only very early in the natural history of the disease. A cohort study spanning decades would be needed to assess this possibility definitively. Cross-sectional studies of fecapentaene excretion in patients with preinvasive colorectal neoplasia of increasing severity would be useful as a practical alternative.

If fecapentaene excretion is truly lower in individuals who develop colorectal cancer, as suggested by our case-control data, the biological implications are unclear. We have shown in an autopsy study that excreted fecapentaene levels are representative of bowel contents throughout the colon (2), but very little is known about the relationship of fecapentaenes in the colonic lumen to tissue-bound levels. Perhaps fecapentaene excretion represents a protective mechanism against colorectal damage, but more information is needed about the production, metabolism, tissue binding, and animal carcinogenicity of fecapentaenes before we can judge the plausibility of this hypothesis. Tissue-binding studies and animal carcinogenicity experiments are underway in several laboratories. Continuing studies of other fecal mutagens will also clarify if fecal mutagenicity generally decreases the risk of colorectal cancer, increases risk as suggested by the early correlational studies, or is unimportant.

Two caveats arise from our investigation. The first concerns patient participation in studies requiring stool collection. It was apparent from the low participation rates among our eligible cases and controls that repeated stool collection is difficult to achieve. Repeated measurements appear to be useful to typify correctly the excretion patterns of individuals, but confirmatory studies should probably aim for increased participation by decreasing the number of stool collections requested. We are hopeful that the low participation rates did not seriously affect our case-control comparison, since the conclusions did not vary when participants were stratified by the number of samples they collected, or when subjects with incomplete participation were excluded.

As a final caution, it seems that any case-control study of fecal constituents and colorectal cancer must consider carefully the possible effects of diagnostic workup, bleeding, surgery, and recovery on the assay results. It is unclear why fecapentaene levels varied during follow-up, even among controls, but our investigation clearly indicates that at least some clinical procedures can be important confounding influences on fecal measurements. Since it is laborious to accumulate relevant stool samples to address these methodological concerns, we offer our stored specimens to investigators embarking on related projects. We currently are examining other fecal mutagens, bile acids, and neutral sterols using the lyophilized stools and the same methodological approach.

REFERENCES

1. Hirai, N., Kingston, D. G. I., Van Tassell, R. L., and Wilkins, T. D. Structure elucidation of a potent mutagen from human feces. J. Am. Chem. Soc., *104:* 6149–6150, 1982.

2. Schiffman, M. H., Bitterman, P., Viciana, A. L., Schairer, C., Russell, L., Van Tassell, R. L., and Wilkins, T. D. Fecapentaenes and their precursors throughout the bowel—results of an autopsy study. Mutat. Res., *208:* 9–15, 1988.
3. Van Tassell, R. L., and Wilkins, T. D. The precursors of fecapentaenes—a preliminary report. Ann. Ist. Super. Sanita, *22:* 933–942, 1986.
4. Dion, P., and Bruce, W. R. Mutagenicity of different fractions of extracts of human feces. Mutat. Res., *119:* 151–160, 1983.
5. Goggelmann, W., Maier, F. K., and Pfaendler, H. R. Mutagenicity of synthetic racemic fecapentaene-12. Mutat. Res., *174:* 165–167, 1986.
6. Plummer, S. M., Grafstrom, R. C., Yang, L. L., Curren, R. D., Linnainmaa, K., and Harris, C. C. Fecapentaene-12 causes DNA damage and mutations in human cells. Carcinogenesis (Lond.), *7:* 1607–1609, 1986.
7. Curren, R. D., Putman, D. L., Yang, L. L., Haworth, S. R., Lawlor, T. E., Plummer, S. M., and Harris, C. C. Genotoxicity of fecapentaene-12 in bacterial and mammalian cell assay systems. Carcinogenesis (Lond.), *8:* 349–352, 1987.
8. Hinzman, M. J., Novotny, C., Ullah, A., and Shamsuddin, A. M. Fecal mutagen fecapentaene-12 damages mammalian colon epithelial DNA. Carcinogenesis (Lond.), *8:* 1475–1479, 1987.
9. Schmid, E., Bauchinger, M., Braselmann, H., Pfaendler, H. R., and Goggelmann, W. Dose response relationship for chromosome aberrations induced by fecapentaene-12 in human lymphocytes. Mutat. Res., *191:* 5–7, 1987.
10. Ehrich, M., Aswell, J. E., Van Tassell, R. L., and Wilkins, T. D. Mutagens in the feces of 3 South-African populations at different levels of risk for colon cancer. Mutat. Res., *64:* 231–240, 1979.
11. Mower, H. F., Ichinotsubo, D., Wang, L. W., Mandel, M., Stemmermann, G., Nomura, A., Heilbrun, L., Kamiyama, S., and Shimada, A. Fecal mutagens in two Japanese populations with different colon cancer risks. Cancer Res., *42:* 1164–1169, 1982.
12. Reddy, B. S., Sharma, C., Darby, L., Laakso, K., and Wynder, E. L. Metabolic epidemiology of large bowel cancer: fecal mutagens in high and low risk population for colon cancer—a preliminary report. Mutat. Res., *72:* 511–522, 1980.
13. Van Tassell, R. L., Schram, R. M., and Wilkins, T. D. Microbial biosynthesis of fecapentaenes. *In:* I. Knudsen (ed.), Genetic Toxicology of the Diet, pp. 199–211. New York: Liss, 1986.
14. Baptista, J., Bruce, W. R., Gupta, I., Krepinsky, J. J., Van Tassell, R. L., and Wilkins, T. D. On distribution of different fecapentaenes, the fecal mutagens, in the human population. Cancer Lett., *22:* 299–303, 1984.
15. Schwartz, S., Dahl, J., Ellefson, M., and Ahlquist, D. The "HemoQuant" test: a specific and quantitative determination of heme (hemoglobin) in feces and other materials. Clin. Chem., *29:* 2061–2067, 1983.
16. Zar, J. H. Biostatistical Analysis. Englewood Cliffs, NJ: Prentice-Hall, Inc., 1974.
17. Schiffman, M. H. Diet and faecal genotoxicity. Cancer Surv., *6:* 653–672, 1987.
18. Taylor, P. R., Schiffman, M. H., Jones, D. Y., Judd, J., Schatzkin, A., Nair, P. P., Van Tassell, R. L., and Block, G. Relation of changes in amount and type of dietary fat to fecapentaenes in premenopausal women. Mutat. Res., *206:* 3–9, 1988.
19. Correa, P., Paschal, J., Pizzolato, R., Pelon, W., and Lesley, D. E. Fecal mutagens and colorectal polyps: preliminary report of an autopsy study. Banbury Rep., *7:* 119–127, 1981.